# Arginine to glutamine mutation in olfactomedin like 3 (*OLFML3*) is a candidate for severe goniodysgenesis and glaucoma in the Border Collie dog breed.

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Abstract

Goniodysgenesis is a developmental abnormality of the anterior chamber of the eye. It is generally considered to be congenital in dogs (*Canis lupus familiaris*), and has been associated with glaucoma and blindness. Goniodysgenesis and early-onset glaucoma initially emerged in Border Collies in Australia in the late 1990s and has subsequently been found in Europe and the USA. The objective of the present study was to determine the genetic basis of goniodysgenesis in Border Collies. Clinical diagnosis was based on results of examinations by veterinary ophthalmologists of affected and unaffected dogs from eleven different countries. Genotyping using the Illumina high density canine SNP chip and whole genome sequencing were used to identify candidate genetic regions. Expression profiles and evolutionary conservation of candidate genes were assessed using public databases. Analysis of pedigree information was consistent with an autosomal recessive mode of inheritance for severe goniodysgenesis (potentially leading to glaucoma) in this breed. There was a highly significant peak of association over chromosome 17, with a *p*-value of $2 \times 10^{-13}$. Whole genome sequences of three dogs with glaucoma, three severely affected and three unaffected dogs identified a missense variant in the olfactomedin like 3 (*OLFML3*) gene in all six affected animals. This was homozygous in all nine cases with glaucoma and 12 of 14 other severely affected animals. Of 67 reportedly unaffected animals, only one (offspring of two homozygous affected parents) was homozygous for this variant. The identification of a candidate genetic region and putative causative mutation will aid breeders to reduce the frequency of goniodysgenesis and the risk of glaucoma in the Border Collie population.

53 **Introduction**

54 Companion animals including cats, dogs and horses, suffer from a range of diseases, with
55 both genetic and environmental aetiologies. The breed barrier created by registration
56 requirements and breeding practices seeking to maximise compliance of animals with breed
57 specifications, results in increased levels of matings between relatives [1] and means that
58 purebred companion animals are particularly likely to suffer from recessive genetic
59 conditions. Understanding the genetic factors underlying these diseases is important to
60 improve welfare within breeds and the strong linkage disequilibrium in such inbred
61 populations means that small numbers of pedigree animals can be used to identify genetic
62 regions of interest, making them excellent genetic models if the same disease is present in
63 humans.

64 In response to the identification of causal variants, breeding strategies can be put in place to
65 reduce the prevalence of conditions that impact on animal welfare, especially when a
66 genetic test can be developed. However, the success of these approaches depends on
67 understanding the mode of inheritance and level of genetic contribution to the disease, and
68 ideally on identifying a causative mutation that can be used to develop a test for genetic
69 status.

70 Primary glaucoma is a condition in which increased ocular pressure damages the retinal
71 ganglion, leading to blindness (reviewed in [2]). In dogs (*Canis lupus familiaris*) it can be
72 preceded by goniodysgenesis (also known as mesodermal dysgenesis), a developmental
73 abnormality of the eye characterised by narrowing or closure of the iridocorneal angle
74 through which the aqueous humour drains. This is associated with alterations in the
75 structure of the pectinate ligament which traverses the drainage angle, called pectinate

76 ligament dysplasia (PLD) [3] (more properly pectinate ligament abnormality [4]). In the
77 Leonberger dog breed, goniodysgenesis has been associated with an increased risk of
78 developing primary closed angle glaucoma (PCAG) [2] and in Flat-Coated Retrievers,
79 development of glaucoma is positively and significantly related to the severity of
80 goniodysgenesis [5]. Goniodysgenesis is generally considered to be congenital in dogs,
81 although its subsequent progression is different among breeds. The severity is thought to
82 increase with age in Leonbergers [2] and Flat-Coated Retrievers [6] and was shown to be
83 strongly associated with age in Dandie Dinmont Terriers, Basset Hounds, Flat-Coated
84 Retrievers, Hungarian Vizslas and Golden Retrievers [7, 8]. However, goniodysgenesis
85 remained stable in Samoyeds [9] and was not associated with age in a small study of Border
86 Collies [8].

87 Sudden onset glaucoma leading to blindness with bilateral loss of eyes began to be seen in
88 young Border Collies in Australia about 15 years ago. A similar, sudden onset glaucoma in
89 young animals subsequently emerged in the UK Border Collie population, often in dogs that
90 were related to the affected Australian dogs. A number of dogs in the USA and Europe have
91 now also been diagnosed with severe goniodysgenesis and glaucoma. Goniodysgenesis in
92 Border Collies has been added to the Schedule B list of "Conditions Under Investigation" in
93 the British Veterinary Association (BVA) Eye Scheme (http://www.bva.co.uk/Canine-Health-
94 Schemes/Eye-scheme/) and it is highly prevalent in some Border collie lineages (see
95 Anadune Border collie database: http://www.anadune.com/; free access but registration
96 required). In a recent study [8], 11 of 102 Border Collies (10.8%) were reported to have
97 moderate or severe PLD (associated with goniodysgenesis). Seven (6.9%) were mildly
98 affected. Because of the association with a small number of popular sires and the high
99 prevalence in some lineages, a genetic aetiology is strongly suspected in Border Collies, but

100 the heritability and mode of inheritance of the condition is currently unknown. It is also not
101 known why a proportion of dogs diagnosed with severe goniodysgenesis go on to develop
102 glaucoma, but some do not, and nor do those with mild goniodysgenesis, over a life span of
103 15 or more years.

104 The aim of the current study was to perform a genome-wide analysis to find genetic regions
105 that are associated with severe goniodysgenesis and glaucoma in the Border Collie and
106 identify candidate mutations that might be responsible for this condition.

107

108 **Materials and Methods**

109 Full details of the Materials and Methods, including details of publicly available data, are
110 given in **Supplemental File S1**.

111 *Ethical approval*

112 All studies were approved by the Veterinary Ethical Review Committee of the University of
113 Edinburgh (approval number VERC 2012-8) and the Animal Ethics Committee of the
114 University of Queensland (approval number ANFRA/MRI-UQ/565/17).

115 *Ascertainment of clinical status and pedigree*

116 Goniodysgenesis was diagnosed by examination of the eyes with gonioscopy to assess the
117 status of the drainage angle and the pectinate ligament fibres that span it. Since we had
118 results in multiple formats from many veterinary ophthalmologists, each report was
119 manually assessed to assign clinical status to each subject. Only individuals who had had
120 gonioscopy analysis were included. Breeding records and pedigree data were obtained from
121 public databases and from the breeders' websites. Websites were accessed at various times
122 up to February 2018. Full details including exclusion criteria are given in **Supplemental File**
123 **S1**.

124 *Genetic analysis*

125 DNA samples were from buccal cells collected by owners and occasionally from blood. Full
126 details of collection and extraction procedures are given in **Supplemental File S1**.
127 Genotyping used the Illumina 173k CanineHD Whole-Genome Genotyping Bead Chip
128 (Illumina, San Diego, CA, USA). Results were filtered in PLINK v1.07 [10] to remove
129 individuals that had more than 10% of missing genotypes and SNPs that had rates of

130 genotyping < 0.95, had minor allele frequency < 0.05 in this population or deviated from the
131 Hardy-Weinberg equilibrium in the controls with P value of less than 0.0001. Only the
132 autosomes were considered.

133 Cases for the analysis were defined as severely affected and consisted of dogs that had
134 goniodysgenesis of grades four or five under an early grading scheme, dogs whose
135 goniodysgenesis was described as "severe", those where the veterinary ophthalmologist
136 report indicated PLD over more than 70% of the iridocorneal angle and those that
137 developed glaucoma following a diagnosis of goniodysgenesis. Controls were dogs that were
138 assessed as clear or clinically unaffected based on at least one gonioscopy examination.
139 Previous work indicated that goniodysgenesis was not progressive in Border Collies [8] so
140 the controls were categorised on the basis of a clear gonioscopy, regardless of age. The SNP
141 analysis was performed in GEMMA v0.94.1 [11] using a linear mixed model approach,
142 controlling for relatedness using a SNP-based relatedness matrix as a random effect. The
143 significance threshold was based on a Bonferroni correction of 0.05. Additionally, a more
144 conservative, regional approach was undertaken using sliding windows of 40 adjacent SNPs,
145 overlapping by 20 SNPs in REACTA v0.9.7 [12] with the first two genomic principal
146 components controlling for relatedness. Whole genome sequencing (30X coverage) of DNA
147 from three dogs with glaucoma, three severely affected and three unaffected animals was
148 performed using the Illumina HiSeq X platform. Library insert sizes were 450 bp and paired
149 reads of 150 bp were sequenced. Reads were processed by standard procedures, detailed in
150 **Supplemental File S1** and mapped to the CanFam3.1 reference genome. The significance of
151 variants was assessed using the Ensembl Variant Effect Predictor [13].

152 *Transcriptomic analysis of candidate region genes*

153 A time course of mouse eye development from embryonic day 12 to adult was examined
154 using the FANTOM5 database [14-16] (http://fantom.gsc.riken.jp/zenbu). FANTOM5 data
155 were also used to obtain expression levels for human eye-related samples (**Supplemental**
156 **Table S1**). Transcriptomic data for the developing chicken eye were generated for another
157 project by RNA sequencing methodology (Rainger et al, in preparation). Details are given in
158 **Supplemental File S1.** Microarray-based expression data for a range of tissues in human and
159 mouse were from BioGPS (http://biogps.org) as were RNA sequencing data for chicken
160 tissues. Microarray expression data for immune cell types was derived from the
161 Immunological Genome Project (https://www.immgen.org/).

162 *Analysis of canine OLFML3 gene*

163 The region of the canine *OLFML3* gene containing the candidate causative mutation was
164 amplified by the polymerase chain reaction and the amplicon sequenced by chain
165 termination sequencing. Full details of primers and protocols are given in **Supplementary**
166 **File S1**. Sequences for analysis of nucleotide and amino acid conservation across species
167 were downloaded from Ensembl (http://ensembl.org) and alignment was performed using
168 Clustal Omega (http://www.ebi.ac.uk/Tools/msa/clustalo/).

169

170 **Results**

171 *Familial clustering of goniodysgenesis and glaucoma*

172 Inspection of pedigrees revealed that there were three common ancestors in both the sire
173 and dam lineages for all cases of glaucoma on the goniodysgenesis database (https://bc-
174 glaucomadatabase.synthasite.com/) (N=12) including two pairs of full siblings both affected
175 with glaucoma, as well as additional cases not on the database (N=4). These common
176 ancestors dated back to the late 1970s and early 1980s and were found in multiple ancestral
177 lineages for the glaucoma cases. All the glaucoma cases had been diagnosed with
178 goniodysgenesis and subsequently had one or both eyes removed. However, several
179 individuals diagnosed with severe goniodysgenesis had not gone on to develop glaucoma.
180 This included two popular sires who reached the age of 15 years without a diagnosis of
181 glaucoma. It also included a full sibling of two dogs with glaucoma. Three dogs with severe
182 goniodysgenesis had produced a total of six offspring with glaucoma.

183 *Genome wide association analysis*

184 To identify genetic regions associated with goniodysgenesis in this breed, we performed
185 SNP analysis on 17 severely affected cases (8 female, 9 male) and 42 unaffected dogs (26
186 female, 16 male) who had been passed as "clear" at veterinary ophthalmology testing. The
187 cases all had goniodysgenesis described as "severe" (N=4), Grade 4 or 5 by the earlier
188 grading scheme (N=1 and 5 respectively), or goniodysgenesis followed by glaucoma (N=7).
189 A strong association of goniodysgenesis with a region of chromosome 17 was found (**Figure**
190 **1A**). The lowest *p*-value was for SNP rs22561716 at 17:51,919,221 ($P=2 \times 10^{-13}$) included in a
191 window of 40 SNPs that had a *p*-value of $3 \times 10^{-07}$ according to REACTA. SNPs with the lowest
192 *p*-values were in a region of about $1 \times 10^{6}$ base pairs (**Figure 1B**). Sixteen of the severely

193 affected and glaucoma cases were homozygous for this haplotype and one case was
194 heterozygous in this region. Within the genomic region identified by the association analysis
195 there were a number of coding genes (**Figure 1A)**, although none was known to be involved
196 in eye development or glaucoma in animals or humans. Several had strong expression in the
197 developing mouse eyeball (**Supplemental Figure F1**).

198 *Whole genome sequencing*

199 We then obtained the whole genome sequence of this region in three glaucoma, three
200 severely affected and three unaffected individuals, who were members of four nuclear
201 families (**Supplemental Figure F2**). All were distantly related through multiple lineages in
202 the extended pedigree. Relatives were chosen to highlight variants that were different
203 between affected and unaffected family members [17]. Within the region of the lowest *p*-
204 value SNP, a missense variant was found in the olfactomedin like 3 (*OLFML3*) gene, at
205 position 17:51,786,924. The mutation was c.590G>A with the predicted change of arginine
206 at position 197 to glutamine (p.R197Q). This arginine is in the olfactomedin (OLF) domain
207 [18, 19] (**Figure 2A**) and was found to be highly conserved throughout mammals, birds and
208 reptiles (**Figure 2B**). The impact predicted by the Ensembl Variant Effect Predictor [13] was
209 given as "moderate" indicating "a non-disruptive variant that might change protein
210 effectiveness", consistent with the non-lethal phenotype of homozygous animals. The SIFT
211 score was 0.55, indicating a mild effect. In humans, the mutation (c.587G>A) corresponds
212 with rs377336789 (https://www.ncbi.nlm.nih.gov/snp/) and was seen in four out of 121,412
213 chromosomes with a minor allele frequency of $3.3 \times 10^{-5}$. Genotypes are not provided for
214 the human data, but these were presumably all heterozygous, given the minor allele
215 frequency. SIFT score was 0.54 and PolyPhen score was 0.392, also consistent with a mild

216 effect. The mutation was heterozygous in four of 35 Border Collies from the Dog Biomedical
217 Variant Database Consortium (giving an allele frequency of 0.06 in this breed) but absent in
218 504 genotypes of other breeds. Notably, it was not present in 51 Leonbergers, three Basset
219 Hounds, eight Golden Retrievers or one Dandie Dinmont Terrier, all breeds reported to be
220 associated with goniodysgenesis. In addition, 350 dogs recorded as Border Collies but of
221 unknown clinical status were genotyped by Animal Genetics (Talahassie, Florida, USA) via
222 allele-specific PCR run on an ABI 3730 machine. There were 2 homozygotes and 29
223 heterozygotes among these 350 dogs, giving an allele frequency of 0.05, consistent with the
224 smaller sample from the Consortium.

225 *OLFML3 genotypes of severely affected and unaffected Border Collies*

226 The results for the mutation c.590G>A in glaucoma, severe and unaffected dogs are shown
227 in Table 1. The individual who was heterozygous for the region based on SNP chip was also
228 AG at *OLFML3*. This animal had been diagnosed with severe goniodysgenesis but did not
229 develop glaucoma over a 15-year lifespan. In addition, one unaffected dog was AA
230 genotype. This animal was the offspring of a severely affected (grade 5) father and a mother
231 diagnosed as affected (with no grade). Both parents were also AA. Under some testing
232 schemes animals could pass with some degree of goniodysgenesis [2, 4] which may be the
233 case with this dog. In general, the genotype for *OLFML3* segregated with that for
234 rs22561716, indicating strong linkage disequilibrium between them. However, an
235 unaffected mother and son were homozygous for G at *OLFML3* but heterozygous at
236 rs22561716 suggesting that the ancestral haplotype was G at *OLFML3* with T at rs22561716.
237 In addition, two unaffected individuals were homozygous for several SNPs either side of

238 *OLFML3* but heterozygous for the *OLFML3* mutation and rs22561716 and appeared to carry
239 a different haplotype.

240 To validate these results, we genotyped 31 additional Border Collies that had had
241 gonioscopy investigations: two with glaucoma (both female), four severely affected (all
242 female) and 25 unaffected (10 male, 15 female). The genotypes of these dogs are shown in
243 Table 1 and are consistent with the results suggesting that severe
244 goniodysgenesis/glaucoma was associated with homozygosity for the A allele of *OLFML3*.

245 *OLFML3 genotypes in mildly affected and uncategorised affected Border Collies*

246 Our initial analysis compared severely or glaucoma affected dogs with dogs passed as
247 unaffected at gonioscopy examination. Because of changes in the reporting scheme in the
248 UK and differences among countries, there were a number of equivocal diagnoses. These
249 were dogs where either the level of goniodysgenesis was given as mild (Grade 1 under the
250 former scheme or stated as mild or marginal on the report; N = 9; five female, four male),
251 moderate (Grade 2 – 3 under the old scheme, or stated as moderate or between 25 and
252 70% blocked on the report; N = 9; eight female, one male), or the dogs were diagnosed as
253 affected with no description given (N = 15; nine female, six male). To examine whether
254 *OLFML3* was involved in these cases we genotyped these 33 dogs. The results are shown in
255 Table 2. All three genotypes were found among these cases.

256 *Expression of OLFML3 in the eye*

257 In expression data generated by the FANTOM5 project, the *Olfml3* gene was strongly
258 expressed in mouse eyeball at embryonic and neonatal stages and declined in the adult
259 sample (**Figure 3A**). FANTOM5 data showed that the gene was expressed in human lens,

260 corneal epithelium and retina (**Figure 3B**), although the highest expression was in amniotic
261 and placental epithelial cells and in mesenchymal stem cells (not shown). *OLFML3* was also
262 expressed in the developing chick eye, with highest expression at embryonic day 6 (**Figure**
263 **3C**). In mouse microarray data (mouse probeset 1448475_at; http://biogps.org) expression
264 of *Olfml3* was highest in osteoblasts undergoing calcification, but it was also found in
265 eyecup, iris, ciliary bodies and lens (**Supplemental Figure S3A**). There was negligible
266 expression in immune system cells, except for macrophages, osteoclasts and particularly
267 microglia, which had expression comparable to lens and eyecup. Strong expression in
268 microglia was also seen in the Immunological Genome data set (**Supplemental Figure S3B**).
269 Microarray data also available through BioGPS showed strongest expression of *OLFML3* in
270 human adipocytes, uterus and retina, the only eye tissue represented (human probeset
271 218162_at; http://biogps.org) (**Supplemental Figure S3C**). RNA sequencing data compiled
272 on BioGPS revealed expression in many samples from the chicken, notably the whole
273 embryo and connective tissues and the cornea of the adult (**Supplemental Figure 3D**). Other
274 OLF family members were also expressed in the developing mouse eyeball with a similar
275 pattern to *Olfml3*, peaking in the early neonatal period and declining in the adult eye
276 (**Supplemental Figure S4**). In contrast, the myocilin gene (encoding a related protein which
277 is mutated in human open angle glaucoma) had negligible expression in embryo and early
278 neonate but increased expression at neonatal day 16 and in adult eyeball (**Supplemental**
279 **Figure S4**).

280

281 **Discussion**

282 In a number of dog breeds development of glaucoma has been associated with the presence
283 of goniodysgenesis. In the Flat-Coated Retriever, the likelihood of glaucoma was positively
284 and significantly related to the severity of goniodysgenesis [5] and in the Leonberger five
285 glaucoma-affected individuals all had a high grade of goniodysgenesis in the contralateral
286 eye [2]. An abnormal drainage angle is considered typical of some breeds, with reports of up
287 to 81% of individuals showing evidence of goniodysgenesis [9, 20]. However, the highest
288 prevalence of glaucoma reported in any single breed is only 5.52% [21], indicating that
289 severe goniodysgenesis does not necessarily proceed to glaucoma.

290 An increasing incidence of glaucoma in young animals has been reported in the Border
291 Collie breed over the last 15 years. Consistent with results in other breeds, our survey of
292 goniodysgenesis in the Border Collie found some individuals who lived entire lifetimes with
293 apparently severe goniodysgenesis without developing glaucoma. This would suggest that
294 although severe goniodysgenesis is important in the progression to glaucoma, it is not the
295 sole cause. The aetiology may involve a genetic predisposition to goniodysgenesis combined
296 with genetic, environmental or random factors influencing development of glaucoma.

297 The diagnosis of goniodysgenesis was made by veterinary ophthalmologists (such as
298 members of the BVA Eye Panel in the UK or the equivalent certifying body in other
299 countries) who performed gonioscopies to assess the status of the drainage angle and the
300 pectinate ligament fibres that cross it. Since the diagnoses were made by a number of
301 different clinicians over a period of years, there may be considerable noise in the
302 phenotype. An initial eye scheme developed by the BVA in association with the Kennel Club
303 of the United Kingdom involved assessing whether the dog had no eye abnormality (a pass),

304 or some abnormality graded from one to five according to the extent of PLD and the width
305 of the iridocorneal angle, in increasing levels of severity. Since there was a high degree of
306 inter-observer variability in assigning a grade, the scheme was subsequently amended such
307 that the dog would either pass or fail. Dogs that passed could have a drainage angle that
308 was abnormal to some extent ("mild" goniodysgenesis; see information available at
309 https://www.bva.co.uk/Canine-Health-Schemes/Eye-scheme/). The pass/fail scheme was
310 expected to have a higher level of inter-observer agreement. This was confirmed in a recent
311 study of Leonberger dogs [2] where there was a high correlation in proportion affected with
312 goniodysgenesis between a prospective study where examinations were performed by a
313 single specialist (2012-2014; 18% affected) and a retrospective study of BVA eye scheme
314 certificates (2009-2014; 22% affected). In a study of PLD in Welsh Springer Spaniels, there
315 was a good correlation between two examiners in determining unaffected eyes, although
316 there was considerable variability between examiners in assigning the degree of PLD [4].
317 This variability in categorising the extent of goniodysgenesis means that the classification of
318 phenotype as "affected" or "unaffected" can result in mild PLD or angle narrowing being
319 recorded as clinically unaffected [2, 4]. For this reason, we focussed our genome-wide
320 analysis on severely affected dogs including several that had gone on to develop glaucoma.

321 Using severely affected cases and unaffected controls, we found a strong peak of
322 association on chromosome 17, in the region of the *OLFML3* gene, where we detected a
323 missense mutation p.R197Q. Although this gene has not been implicated in goniodysgenesis
324 or glaucoma in humans, it is strongly expressed in tissues of the anterior segment of the
325 human and baboon eye including lens, iris, sclera and trabecular meshwork [18, 22] and in
326 the developing mouse eye [23] . The *Olfml3* gene was expressed throughout mouse eyeball
327 development (**Figure 3A**) and during chick eye development (**Figure 3C**). In mouse,

328 inactivation of the *Olfml3* gene by insertion of a *Lac* cassette resulted in viable and fertile
329 homozygotes [23], consistent with the relatively mild selective disadvantage of dogs with
330 goniodysgenesis. Expression has also been observed in human tissues of mesenchymal
331 origin such as bone and adipose. Olfactomedin like 3 is an extracellular matrix protein that
332 has been implicated in the epithelial to mesenchyme transition in cancer [24], in keeping
333 with its mesenchymal expression and suggesting that abnormality of this protein could be
334 associated with the formation or retention of abnormal sheets of mesenchyme in the
335 drainage angle. Olfactomedin like 3 is also proangiogenic [25] and interacts with a member
336 of the bone morphogenic protein (BMP) family, BMP4. Since BMPs including BMP4 are
337 involved in eye development (see [26] for a review), this may indicate the pathway for
338 olfactomedin like 3 action.

339 In addition, expression of *OLFML3* is also characteristic of microglia in humans [27, 28] and
340 mice (http://biogps.org; http://www.immgen.org), although it is not strongly expressed by
341 other macrophage lineages. Genetic manipulation resulting in absence of microglia is
342 associated with loss of *Olfml3* expression in mice (R Rojo et al, in revision). The retina
343 contains microglia which are important for retinal health [29, 30], and they could be one
344 source of *OLFML3* mRNA in the canine eye. Opening of the iridocorneal angle involves
345 considerable remodelling of the mesenchymal tissue within the angle, including thinning
346 and extending of the pectinate ligament and opening the trabecular meshwork [31].
347 Microglia may contribute to this process as eye development progresses by phagocytosing
348 apoptotic cells and debris from the remodelling. Abnormality of olfactomedin like 3 in the
349 microglia of the eye could interfere with this function and therefore disrupt the opening of
350 the iridocorneal angle and formation of the proper drainage channels.

351 Olfactomedin like 3 is a member of a large family characterised by the olfactomedin (OLF)
352 domain [19, 32], with several members expressed in eye structures. In particular, the
353 glucocorticoid-inducible family member myocilin (*MYOC* gene) has been implicated in
354 dominant open angle glaucoma in humans [33] and is strongly expressed in the trabecular
355 meshwork of the drainage angle [34, 35]. As with *Olfml3* mutants, there is no gross
356 phenotype in *Myoc* knockout mice [36]. Increased expression of *Myoc* mRNA resulted in a
357 reduction in *Olfml3* mRNA [37] in a mouse model, suggesting that there is an inverse
358 association between these two OLF proteins, consistent with the expression profiles in the
359 developing mouse eye (**Supplemental Figure S4**) where *Olfml3* declined in the adult while
360 *Myoc* rose. *In vitro* experiments indicated that myocilin interacts with olfactomedin 3 (also
361 known as optimedin, encoded by the *OLFM3* gene) which was co-expressed with myocilin in
362 the eye [38]. Mutation in another OLF family member, *OLFM2,* has been associated with
363 human open angle glaucoma in a small number of Japanese patients (rs779032127;
364 p.R144Q) [39] and identified as contributing to eye development [40]. These other OLF
365 family members may compensate for olfactomedin like 3 abnormalities, or may interact
366 with olfactomedin like 3 in the development of the eye. Hence genetic variation in these
367 family members may modify the impact of the *OLFML3* mutation in the Border Collies,
368 explaining the variable phenotype of homozygotes and heterozygotes carrying the
369 mutation.

370 We propose that the p.R197Q mutation in Border Collies is responsible for the presence of
371 severe goniodysgenesis predisposing to glaucoma in homozygous animals. We have also
372 seen some heterozygous animals with mild to relatively severe phenotypes (although none
373 with glaucoma), but most heterozygotes have been passed as clinically unaffected at
374 gonioscopy testing. This suggests that there may be variants at modifier loci that can

375 compensate for the *OLFML3* mutation. In particular, the presence of many OLF proteins in
376 the eye and the mild phenotype associated with relatively broadly expressed gene family
377 members suggests redundancy for at least some functions. It is also possible that
378 inadequate compensation for the putative effects of the p.R197Q mutation may be
379 responsible for the progression from severe goniodysgenesis to glaucoma.

380 There were too few glaucoma cases in our study to allow identification of loci involved in
381 progression from goniodysgenesis to glaucoma in the Border Collie and ambiguous
382 phenotyping complicates the investigation of mild goniodysgenesis. The genetic relationship
383 between the mild forms and the more severe forms is unknown, but the *OLFML3* variant
384 described here does not appear to be associated with most cases of mild goniodysgenesis
385 indicating that genetic predisposition to mild goniodysgenesis may be independent of the
386 findings of this study. Although developing a test for the *OLFML3* mutation would allow
387 breeders to select against homozygotes to decrease the prevalence of the risk genotype for
388 severe goniodysgenesis, it is important not to reduce the breeding pool too much because
389 of the risk of other recessive conditions resulting from homozygosity for alleles that are
390 identical by descent. Therefore, we need to understand why some of our cases progressed
391 to glaucoma at a young age and some did not before we can make recommendations to the
392 breeders of Border Collies worldwide.

393

394 **Author contributions**

395 KMS, CAP and LLF conceived the study. AJC, LLF and KMS curated and prepared the DNA
396 samples. OM, SCB and LLF performed initial genetic analysis. LLF and CAP performed the
397 SNP analysis. CAP and SJB performed the genome wide sequencing analysis. KMS and AJC

398 designed, performed and analysed the *OLFML3* genotyping. JJS analysed the data from the
399 Dog Genome Consortium. AdeK and CW analysed the Animal Genetics samples. KMS
400 analysed the public gene expression data. JR and VT-R created and analysed the chicken
401 eye data. KMS, LLF and CAP drafted the text and all authors read and edited the final
402 manuscript.

403

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415 supporting the project.

416

417 **Conflict of Interest**

418 AdeK and CW are employed by Animal Genetics, Talahassie, FL, USA, which is now offering a
419 test for genetic predisposition to severe goniodysgenesis based on these results.

420

421 **Figure legends**

422 **Figure 1.** Genome wide association with severe goniodysgenesis and glaucoma

423 **A.** Manhattan plot for the canine genome; chromosome 17 is shown in more detail and
424 genes in the region are shown below.

425 **B.** Homozygosity within the region of interest. Each row is a DNA sample and each column
426 shows a SNP. Red and yellow indicate homozygosity for the major and minor allele
427 respectively and green shows heterozygosity. The upper block shows severely affected and
428 glaucoma animals; the lower block shows those passed as clinically unaffected at
429 gonioscopy testing. The most significant SNP is shown as yellow in the cases (homozygous
430 for the minor allele); one clinically unaffected animal was homozygous for this allele and
431 one severely affected animal was heterozygous.

432

433 **Figure 2.** Conservation of *OLFML3* predicted amino acid sequence

434 **A.** Amino acid sequence of the canine OLFML3 protein. Functional domains are highlighted:
435 blue – signal peptide; pink – coiled coil domain; yellow – olfactomedin like domain [18, 19].
436 Within the yellow region, bold lettering shows the core OLF region. Green shows the
437 arginine at position 197.

438 **B.** Conservation of arginine 197 (green) in OLFML3 of vertebrates. Arginine is found at the
439 equivalent position in all mammal, bird and reptile species for which there is an annotated
440 *OLFML3* gene, and in coelacanth but not in other fish. The region around this arginine is also
441 highly conserved.

442

443 **Figure 3.** Expression of *OLFML3* genes in eye regions

444 **A.** Expression of *Olfml3* in developing mouse eyeball. Results are based on CAGE and were
445 downloaded from the FANTOM5 database (http://fantom.gsc.riken.jp/5/tet). Y axix shows
446 RLE normalised values. Light grey – embryonic samples; dark grey – neonatal samples; black
447 – adult sample.

448 **B.** Expression of *OLFML3* in human eye tissues and cells. Results are based on CAGE and
449 were downloaded from the FANTOM5 database. Y axis shows RLE normalised values. Light
450 grey – optic nerve and retina; mid grey – cornea; dark grey – lens.

451 **C.** Expression of *OLFML3* in the developing chick eye. Results are based on RNA sequencing.
452 Y axis shows transcripts per million (TPM) values. Light grey – embryonic day 5; mid grey –
453 embryonic day 6; dark grey – embryonic day 7.

454

455 **Supplemental Material**

456 **Supplemental Table S1.** FANTOM5 samples used in analysis of gene expression. Details can
457 be found at fantom.gsc.riken.jp/5/sstar/.

458 **Supplemental Figure S1**. Expression of genes in the candidate region in the developing
459 mouse eye. Upper panel shows the canine genes in the region. Lower panel shows
460 expression of the genes in the developing mouse eyeball. Y axis shows RLE normalised
461 expression levels. Data taken from FANTOM5 (see **Supplemental Table S1**). Light grey –
462 embryonic; dark grey – neonatal; black – adult.

463 **Supplemental Figure S2.** Pedigrees of animals used for whole genome sequencing. Grey fill
464 indicates severe goniodysgenesis; black fill indicates glaucoma; white fill indicates
465 unaffected or unknown status. Double line indicates a first cousin mating; all glaucoma
466 individuals can be traced back to the same three distant common ancestors on both sides.
467 Animals with a BC ID were sequenced.

468 **Supplemental Figure S3.** Expression of olfactomedin like 3 gene in cells and tissues

469 A. Expression in mouse tissues and cell lines (http://biogps.org; microarray probeset
470 1448475_at). Y axis shows relative intensity of expression (arbitrary fluorescence
471 units).
472 B. Expression in mouse myeloid cells (http://www.immgen.org, dataset "MFs,
473 Monocytes, Neutrophils"; microarray probeset 10500808). Y axis shows relative
474 intensity of expression (arbitrary fluorescence units).
475 C. Expression in human tissues and cell lines (http://biogps.org; microarray probeset
476 218162_at). Y axis shows relative intensity of expression (arbitrary fluorescence
477 units).
478 D. Expression in chicken tissues (RNA sequencing data curated from public databases
479 and analysed using Kallisto, available at
480 http://biogps.org/dataset/BDS_00031/chicken-atlas/; Bush et al, in revision). Y axis
481 shows tags per million (TPM).

482 In A, C and D, blue – nervous tissues; dark green – connective tissues; light green –
483 digestive tract and renal; yellow – reproductive; orange – immune; red – ocular.

484 **Supplemental Figure S4.** Expression of olfactomedin family genes in the developing mouse
485 eye (data from FANTOM5; http://fantom.gsc.riken.jp/zenbu/). Y axis shows RLE normalised

486 expression levels based on CAGE analysis. Light grey – embryonic; dark grey – neonatal;

487 black – adult.

488

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598

599

600

| Genotype<br><br>Clinical status | AA | AG | GG | TOTAL |
|---|---|---|---|---|
| ***SNP-chip dogs***[1] | | | | |
| **Glaucoma** | 7 | 0 | 0 | 7 |
| **Severe goniodysgenesis** | 9 | 1 | 0 | 10 |
| **Unaffected** | 1 | 19 | 22 | 42 |
| ***Other dogs*** | | | | |
| **Glaucoma** | 2 | 0 | 0 | 2 |
| **Severe goniodysgenesis** | 3 | 1 | 0 | 4 |
| **Unaffected** | 0 | 17 | 8 | 25 |
| **TOTALS** | | | | |
| **Glaucoma** | 9 | 0 | 0 | 9 |
| **Severe goniodysgenesis** | 12 | 2 | 0 | 14 |
| **Unaffected** | 1 | 36 | 30 | 67 |

601

602 **Table 1.** Genotype frequencies for *OLFML3* mutation c.590G>A in severely affected and unaffected

603 dogs.

604 Dogs that were included in the genome wide analysis (SNP-chip dogs) are shown separately from

605 dogs that were only tested for *OLFML3*.

606

| Genotype<br><br>Clinical status | AA | AG | GG | TOTAL |
|---|---|---|---|---|
| | | | | |
| **Mild** | 3 | 3 | 3 | 9 |
| **Moderate** | 4 | 2 | 3 | 9 |
| **Affected, no grade** | 7 | 3 | 5 | 15 |
| **TOTALS** | 14 | 8 | 11 | 33 |

607

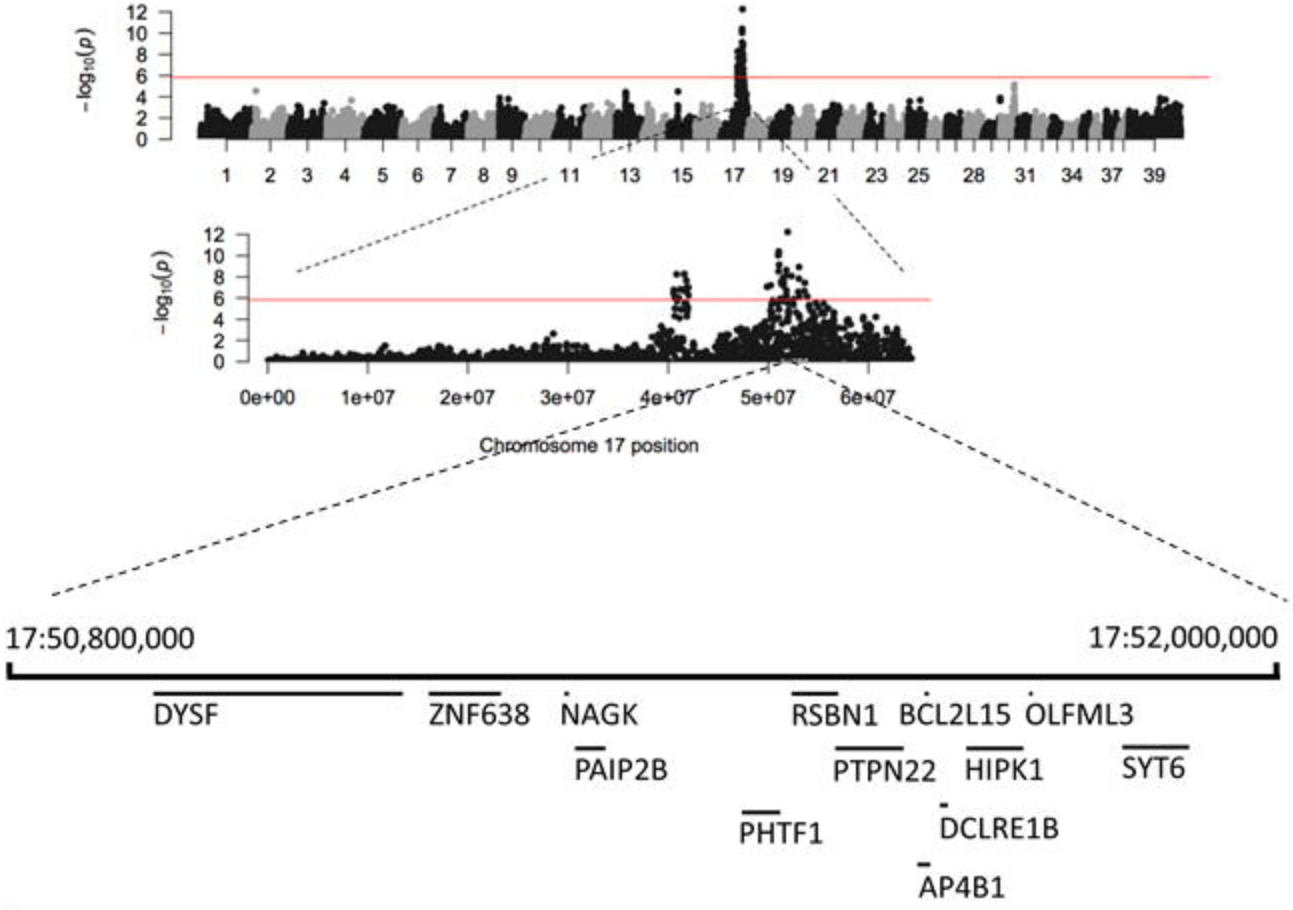
608

609 **Table 2.** Genotype frequencies for *OLFML3* mutation c.590G>A in mild, moderate and

610 uncharacterised affected dogs.

611

A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10
8
6
4
2
0
1 2 3 4 5 6 7 8 9 11 13 15 17 19 21 23 25 28 31 34 37 39
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Chromosome 17 position
17:50,800,000
17:52,000,000
DYSF
ZNF638
NAGK
RSBN1
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PAIP2B
PTPN22
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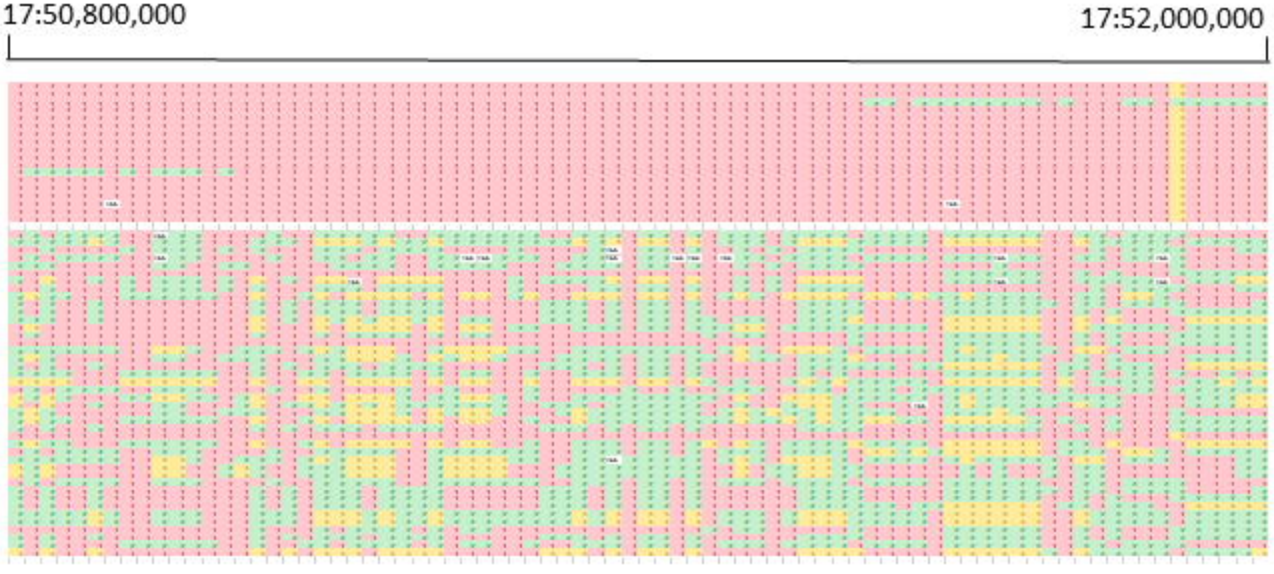
SYT6
PHTF1
DCLRE1B
AP4B1

B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17:52,000,000

**A**

MGPYTPLLTMFLLSWWLRPLQGQQHHLVEYMERRLAALEERLAQCQDQSSRHAAELRDFE

NKMLPLLEVAEKEREALRTEADTISGRVDRLEREVDYLETQNPALPCVEVDEKVTGGPGI

KGKGRRNEKYDMLTDCGYTISQVRSMKILKRFGGPAGLWTKDPMGPTEKIYVLDGTQNDT

AFVFPRLRDFTLAMAARKASRVRVPFPWVGTGQLVYGGFLYYARRPPGGPGGGGGLENTL

QLIKFHLANRTVVDSSVFPAEGLIPPYGLTADTYIDLAADEEGLWAVYATREDDRHLCLA

KLDPQTLDTEQQWDTPCPRENAEAAFVICGTLYVVYNTRPASRARIQCSFDASGTLTPER

AALPYFPRRYGAHASLRYNPRERQLYAWDDGYQIVYKLEMRRKEEEV

**B**

```
Opossum_OLFML3          VRSMKILKRFGSPAGMWTKDPLGPTEKIFVLDGTQNDTAYVFPRLRDFTLSTTARKASRV
Wallaby_OLFML3          VRSMKILKRFGSSAGMWTKDPLGPSEKIFVFDGTQNDTAYVFPRLRDFTLSSAARKASRV
Tasmanian_Devil_OLFML3  VRSMKILKRFGSSAGMWTKDPLGHSEKIFVLDGTQNDTAYVFPRLRDFTLSTAARKASRV
Shrew_OLFML3            VRSMKILKRFGGPTGLWTKDPLGPTEKIFVLDGTHNDTAFIFPRLRDFTLAMAARKAARV
Kangaroo_rat_OLFML3     VRSMKILKRFGGPAGMWTKDPLGPAEKIYVFDGTQNDTALVFPRLRDFTLAMAARKASRI
Hedgehog_OLFML3         VRSMKILKRFGSPPGLWTKDPLGTTEKIYVLD-PQNDTAFIFPRLRDFTLAMAARKASRV
Armadillo_OLFML3        VRSMKILKRFGGPAGLWTKDPLGPAERIYVLAGTQNDTALVFPKLRDFTLATAARKASRV
Mouse_Olfml33           VRSMKILKRFGGSVGLWTKDPLGPAEKIYVLDGTQNDTAFVFPRLRDFTLAMAARKASRI
Rat_Olfml3              VRSMKILKRFGGSAGLWTKDPLGPAEKIYVLDGTQNDTAFVFPRLRDFTLTMAARKASRI
Elephant_OLFML3         VRSMKILKRFGGPAGLWTKDPLGPTEKIYVLDGTQNDTAFVFPRLRDFSLSTATRKASRV
Guinea_pig_OLFML3       VRSMKILKRFGGPAGLWTKDPLGPTEKIYVLDGTQNDTAFVFPRLRDFTLAMAARKASRV
Hyrax_OLFML3            VRSMKILKRFGGPAGLWTKDPLGPAEKIYVLDGTQNDTAFVFPRLRDFSLSMATRKASRV
Microbat_OLFML3         VRSMKILKRFGGSAGLWTKDPLGPTEKIYVLDGTQNDTAFVFPRLRDFTLAMAARKASRV
Squirrel_OLFML3         VRSMKILKRFGGPAGLWTKDPLGPTEKIYVLDGTQNDTAFVFPRLRDFTLTMAARKASRV
Pig_OLFML3              VRSMKILKRFGGPAGLWTKDPLGPTEKIFVLDGTQNDTAFVFPRLRDFTLAMAARKASRI
Horse_OLFML3            VRSMKILKRFGGPAGLWTKDPMGPTEKIYVLDGTQNDTAFVFPRLRDFTLAMAARKASRV
Dog_OLFML3              VRSMKILKRFGGPAGLWTKDPMGPTEKIYVLDGTQNDTAFVFPRLRDFTLAMAARKASRV
Ferret_OLFML3           VRSMKILKRFGGPAGLWTKDPMGPTEKIYVLDGTQNDTAFVFPRLRDFTLAMAARKASRV
Panda_OLFML3            VRSMKILKRFGGPAGLWTKDPMGPTEKIYVLDGTQNDTAFIFPRLRDFTLAMAARKASRV
Cat_OLFML3              VRSMKILKRFGGPAGLWTKDPMGPAERIYVLDGTQNDTAFVFPRLRDFTLAMAARKASRV
Mouse_lemur_OLFML3      VRSMKILKRFGGPAGLWTKDPLGPTEKIYVLDGTQNDTAFVFPRLRDFTLAMAARKASRV
Human_OLFML3            VRSMKILKRFGGPAGLWTKDPLGQTEKIYVLDGTQNDTAFVFPRLRDFTLAMAARKASRV
Cow_OLFML3              VRSMKILKRFGGPAGLWTKDPLGPAEKIYVLDGTQNDTAFVFPRLRDFTLAMAARKASRV
Sheep_OLFML3            VRSMKILKRFGGPAGLWTKDPLGPAEKIYVLDGTQNDTAFVFPRLRDFTLTMAARKASRV
Dolphin_OLFML3          VRSMKILKRFGGPAGLWTKDPLGPAEKIYVLDGTQNDTAFVFPRLRDFTLAMAARKASRV
Coelacanth_OLFML3       IKSMKILKRIGSSTGMWARDPLGDSDNIYVLEGTGNDTVYEYSRARDFTQSFVMR-ARRI
Xenopus_OLFML3          VTAMKILKRFGSSAGLWTKDLAGNSDRIYVFDGAGNDTVYVYPRMKEFTLSSSTRKAAKI
Chicken_OLFML3          VRAMKILKRFGSSAGLWTKDAAGSSEKIYVFDGTANDTVYIFPRMREFTLFSATRRAARI
Flycatcher_OLFML3       VRAMKILKRFGSASGLWTKDAAGSSEKIYVFDGTANDTVYVFPRMREFTLFSATRKAARI
Lizard_OLFML3           VKAMKILKRFGSTAGLWTKDPVGNSEKIYVFDGTSNDTVYVFPRMREFTLFSATRKAARI
Turtle_OLFML3           VKAMKILKKFGSSAGLWTKDPVGSSEKIYVFDGTSNDTVYVFPRMREFTLFSATRKAARI
                         : :*****:*.  *:*::*  * ::.*:*:    ***.  : : ::*:    .*:* ::
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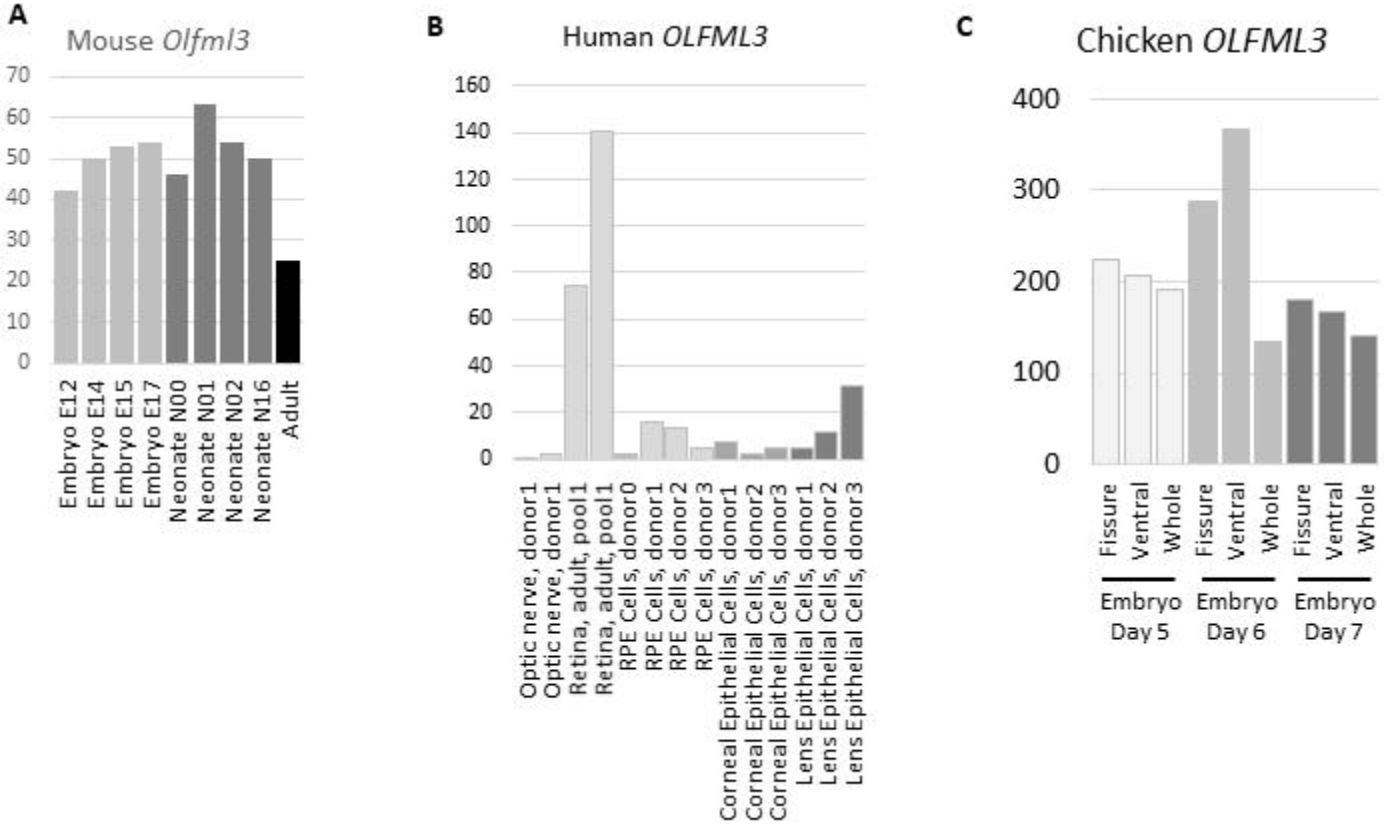
A
Mouse Olfml3
70
60
50
40
30
20
10
0
Embryo E12
Embryo E14
Embryo E15
Embryo E17
Neonate N00
Neonate N01
Neonate N02
Neonate N16
Adult
B
Human OLFML3
160
140
120
100
80
60
40
20
0
Optic nerve, donor1
Optic nerve, donor1
Retina, adult, pool1
Retina, adult, pool1
RPE Cells, donor0
RPE Cells, donor1
RPE Cells, donor2
RPE Cells, donor3
Corneal Epithelial Cells, donor1
Corneal Epithelial Cells, donor2
Corneal Epithelial Cells, donor3
Lens Epithelial Cells, donor1
Lens Epithelial Cells, donor2
Lens Epithelial Cells, donor3
C
Chicken OLFML3
400
300
200
100
0
Fissure
Ventral
Whole
Fissure
Ventral
Whole
Fissure
Ventral
Whole
Embryo Day 5
Embryo Day 6
Embryo Day 7